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Peeling skin, leukonychia, acral punctate keratoses, cheilitis and knuckle pads with milia caused by loss-of-function mutations in calpastatin

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We have identified loss-of-function mutations in the protease inhibitor calpastatin (CAST) gene as the genetic cause of an autosomal recessive skin disease characterised by peeling skin, leukonychia, acral punctate keratoses, cheilitis and knuckle pads with milia (PLACK syndrome). Patient one from a Chinese consanguineous family, presented as an adult with generalised skin peeling and a history of superficial acral blistering in childhood, as well as the features above. Patient two from a Nepalese family developed acral punctate keratoses and cheilitis age 1 year, acral and limb superficial peeling developed age 3 years, as well as leukonychia. Punctate lesions on the dorsum of the hands coalesced into knuckle callosities with milia. Exome sequencing showed that both patients had homozygous loss-of-function mutations. The Chinese patient had a frameshift mutation (c.607_608insAfs, p.I203Nfs*8) and the Nepalese patient had a nonsense mutation (c.A232T, p.K78X). Calpastatin is an endogenous inhibitor of calpain, a calcium-dependent cysteine protease. Immunohistochemistry shows CAST is expressed in the suprabasal layers of normal epidermis but was reduced in patient skin. Transmission electron microscopy (TEM) revealed widening of intercellular spaces in the upper spinous layer in a patient, suggesting loss of intercellular adhesion. A significant increase in apoptotic cells and apoptotic body formation was observed by TUNEL and TEM assays in patient skin. In vitro studies utilising siRNA-mediated CAST knockdown (KD), confirmed by western blotting, revealed a defect in keratinocyte adhesion. In summary, we describe PLACK syndrome caused by loss-of-function mutations in the CAST gene.

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The Cylindromatosis gene product, CYLD, interacts with MIB2 to regulate Notch signalling

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CYLD, an ubiquitin hydrolase, has an expanding repertoire of regulatory roles in cell signalling and is dysregulated in a number of cancers. To dissect CYLD function we used a proteomics approach, and identified MIB2, an ubiquitin ligase enzyme involved in Notch signalling, as a protein which interacts with CYLD. Coexpression of CYLD and MIB2 resulted in stabilisation of MIB2 associated with reduced levels of JAG2, a ligand implicated in Notch signalling. Conversely, silencing of CYLD using siRNA, resulted in increased JAG2 expression and upregulation of Notch signalling. We investigated Notch pathway activity in skin tumours from patients with germline mutations in *CYLD* and found that JAG2 and Notch target genes were upregulated. In particular, RUNX1 protein, a Notch target previously associated with leukaemia, was overexpressed in CYLD defective tumour cells. Finally, primary cell cultures of CYLD defective tumours demonstrated reduced viability when exposed to gamma secretase inhibitors that pharmacologically target Notch signalling. Taken together these data suggest crosstalk between the NF-κB and Notch pathways and may indicate productive novel therapeutic approaches for patients with CYLD defective tumours. Furthermore, the impact of the MIB2-CYLD interaction upon Notch signalling adds to the emerging interactions between CYLD and this oncogenic signalling network.

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Mutations in *POGLUT1* and *POFUT1* cause Dowling-Degos disease and elucidate a gene-phenotype correlation

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Dowling-Degos disease (DDD) is an autosomal dominant genodermatosis characterized by progressive reticulate hyperpigmentation. We had previously identified loss-of-function mutations in *KRT5* in fewer than half of the individuals of our DDD cohort. Recently mutations in *POFUT1* were reported for DDD. Here we describe the identification of a novel gene for DDD. We perfomed exome sequencing on five unrelated individuals with similar DDD phenotypes. Data analysis revealed 3 mutations, all of which are in *POGLUT1*, encoding protein O-glucosyltransferase 1. POGLUT1 and POFUT1 are both involved in posttranslational modification of Notch receptors. By further screening DDD cases, we identified six additional mutations in *POFUT1* and 6 novel mutations in *POGLUT1*. Immunohistochemistry of skin biopsies showed significantly weaker POGLUT1 staining in the upper parts of the epidermis compared to healthy controls. Immunofluorescence analysis identified a co-localization of the wild type protein with the ER and a notable aggregating pattern for the truncated protein. Of interest, a careful analysis of the phenotypes with mutations in the different genes - *KRT5*, *POFUT1* and *POGLUT1* - revealed a correlation between the gene where the mutation is found and the clinical presentation of the disease. Our results emphasize the contribution of Notch pathway to disease pathophysiology and the gene-phenotype correlation in this disorder which is very beneficial for molecular diagnostic efforts.

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Deleterious *AP1S3* alleles are associated with pustular psoriasis but not plaque psoriasis

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The pathogenesis of pustular psoriasis (PP) remains poorly understood, as mutations of the *IL36RN* gene (the only known PP locus) only account for a minority of cases. Here, we undertook whole-exome sequencing in 9 unrelated patients (all suffering from localised disease), with a view to uncovering further genetic determinants for PP. We found that 4 individuals carried a recurrent heterozygous allele (p.Arg33Trp) of the *AP1S3* gene, which encodes a protein involved in intra-cellular vesicular transport. To validate our genetic findings, we screened *AP1S3* coding exons in 119 subjects with localised (n=114) or generalised (n=5) disease. We detected the p.Arg33Trp substitution in 5 affected individuals and uncovered a p.Phe4Cys change in a further 6 cases. In-depth bioinformatics and structural analyses indicated that both variants had clear pathogenic potential. Moreover, the frequency of the damaging alleles was significantly higher in the patient cohort than in the general population, as demonstrated by the analysis of 1,695 unrelated controls ($p=2.5x10^{-7}$). Since 7 of the 14 patients bearing mutations presented with concomitant psoriasis vulgaris (PV), we next sought to determine whether *AP1S3* variants also contribute to PV susceptibility. The analysis of a publicly available ImmunoChip dataset (10,588 psoriasis cases vs. 22,806 controls) showed no evidence of association between *AP1S3* alleles and PV ($p>0.1$). Moreover, the interrogation of preliminary HumanExome chip data did not reveal any enrichment of low frequency *AP1S3* alleles among PS cases. Taken together, these findings identify *AP1S3* mutations as a new genetic determinant for pustular psoriasis and support the notion of a distinct genetic basis for this condition.

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FERMT1 promoter mutations in patients with Kindler syndrome

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Mutations in the *FERMT1* gene, encoding the focal adhesion protein kindlin-1 underlie the KS, an autosomal recessive skin disorder with a phenotype comprising skin blistering, photosensitivity, progressive poikiloderma with extensive skin atrophy, and propensity to skin cancer. *FERMT1* mutational spectrum comprises gross genomic deletions, splice site, nonsense and frameshift mutations, which are scattered over the coding region spanning exon 2 to 15. A single genomic deletion over the putative *FERMT1* promoter sequence and the first noncoding exon has been recently described. We now report three additional KS families with mutations affecting the promoter region of *FERMT1*. Two of these mutations are large deletions (~38.0 and 1.9 kb in size) and one is a single nucleotide variant (c.-20A>G) within the 5′UTR. Each mutation resulted in loss of gene expression in the skin or in cultured keratinocytes from the patients. Reporter assays demonstrated the functional relevance of the genomic regions deleted in our patients for *FERMT1* gene transcription and proved the causal role of the c.-20A>G variant in abrogating transcriptional activity.

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Genome-wide association study identifies three novel susceptibility loci for severe *acne vulgaris*

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Acne vulgaris (acne) is a common complex inflammatory skin disease associated with irreversible scarring. The pathophysiology is centered on the pilosebaceus unit but primary events remain poorly understood. Twin studies show high heritability of 80%, indicating a genetic basis for acne. However, efforts to characterize the genomic risk loci for acne have been limited. Using a national network of dermatologists within the United Kingdom, we recruited subjects with severe acne. Initially, a genome-wide association analysis was performed in cases (n = 1,893, 88% isotretinoin-treated, 28% nodulocystic) and unselected controls (n = 5,132). In a second stage, we genotyped 350 putative associated SNPs in a further 2,063 cases and 1,970 controls with no past or present history of acne. Three genome-wide significant associations were identified: 11q13.1 (rs478304: Pcombined = 3.23 x 10-11, odds ratio (OR) = 1.20), 5q11.2 (rs38055, Pcombined = 4.58 x 10-9, OR = 1.17) and 1q41 (rs1159268: Pcombined = 4.08 x 10-8, OR = 1.17). All three loci contain genes linked to the TGFβ cell signaling pathway, namely OVOL1, FST and TGFB2. Transcripts of OVOL1 (Padjusted = 4 x 10-2) and TFGB2 (Padjusted = 6 x 10-4) were reduced in biopsies of acne lesions compared to uninvolved skin. We could not confirm two association signals close to genes involved in leukocyte adhesion (SELL) and DNA damage response (DDB2) published in a recent Han Chinese GWAS. Collectively, these data support a key role for dysregulation of TGFβ mediated signaling in acne and motivate more in-depth functional studies on the role of TGFβ in acne.

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Hidradenitis suppurativa: Haploinsufficiency of the γ-secretase components NCSTN and PSENEN does not affect γ-secretase enzyme activity *in vitro*

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Hidradenitis suppurativa (HS) is a debilitating inflammatory dermatosis that can be inherited in an autosomal dominant manner. Heterozygous mutations have been reported in the γ-secretase genes *NCSTN*, *PSENEN* and *PSEN1*. The aim of this study was to assess the location of γ-secretase components in the skin and ascertain the molecular mechanisms by which two previously reported mutations (*NCSTN* c.1125+1 G>A, *PSENEN* c.66_67insG) result in HS. Immunohistochemical analysis of axillary skin from unaffected volunteers and mutation-positive patients revealed co-localisation of NCSTN and PSENEN in the epidermis, hair follicle, sebaceous gland, apocrine gland, fibroblasts and inflammatory infiltrates. *NCSTN* c.1125+1 G>A resulted in aberrant splicing and skipping of *NCSTN* exon 9 (predicted protein p.Glu333_Gln367del). *PSENEN* c.66_67insG was predicted to result in a frameshift and an altered protein product (p.Phe23Valfs*98). *NCSTN* and *PSENEN* mRNA expression was reduced in primary dermal fibroblasts harvested from the respective mutation-positive patients vs. healthy volunteers. NCSTN and PEN-2 (encoded by *PSENEN*) protein expression was correspondingly reduced in respective mutant dermal fibroblasts vs. wild type controls. Enzyme activity assays were performed using solubilised cell membrane protein derived from primary human fibroblasts *in vitro*. There was no significant difference in complex number, maturity or endopeptidase or carboxypeptidase-like enzyme activity in samples harvested from mutation-positive patients vs. unaffected volunteers. Overall, these studies revealed that both NCSTN and PSENEN are expressed and co-localise in the skin and that haploinsufficiency of these γ-secretase components underlies some cases of HS. This conferred no demonstrable affect on γ-secretase enzyme activity *in vitro* under the conditions studied.

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Impaired TGFβ signalling and differentiation in Linear morphea fibroblasts which down-regulate SOSTDC1

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Linear morphea(LM) is a skin restricted fibrotic disorder with unknown cause. The disease bears similarity to processes which occur during wound healing, where a TGFβ-rich environment is thought to encourage fibroblast collagen production, activation and differentiation. In this study, fibroblasts were isolated from lesional skin and compared with normal site-matched fibroblasts. Cellular phenotype, transcriptome, and TGFβ signalling kinetics were examined. LM fibroblasts showed altered proliferation, migration and contact inhibition compared to non-lesional fibroblasts. Transcriptome analysis showed changes in TGFβ responsive transcripts, and downregulation of SOSTDC1, a key regulator of TGFβ, Wnt and BMP signalling. A study of 8 lesional skin sections found 50% with SOSTDC1 downregulation (n=8), which was consistent across different sites in the same patient. Analysis of systemic sclerosis lesions showed 40% down-regulated SOSTDC1 (n=5). Basal levels of SMAD phosphorylation were increased in LM fibroblasts, even when serum starved, which paradoxically reduced further induction in response to TGF-β1 stimulus. Consistent with the observed TGFβ dysfunction, ACTA expression, a marker of myofibroblast differentiation, was altered in LM fibroblasts. SOSTDC1 knockdown increased migration, and altered basal SMAD phosphorylation and response to TGF-β1, in a similar fashion to human LM fibroblasts. In conclusion, Linear morphea fibroblasts down-regulate SOSTDC1, which alters cellular migration, contact inhibition and TGFβ signalling. Basal SMAD phosphorylation was increased and LM fibroblasts show altered myofibroblast differentiation. SOSTDC1 downregulation is a common feature of linear morphea disease, suggesting future therapeutic possibilities for the treatment of Scleroderma such as re-introduction of SOSTDC1, or unaffected fibroblasts, to an affected site.

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Skin-specific transgenic Kallikrein 5 mice reproduce major cutaneous and systemic hallmarks of Netherton syndrome : a viable model for therapeutic development

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Netherton syndrome (NS) is a severe genetic skin disease in which absence of LEKTI, a key protease inhibitor causes congenital exfoliative erythroderma, eczematous-like lesions and atopic manifestations. Several proteases are overactive in NS, including kallikrein-related peptidase (KLK)5, KLK7 and elastase-2 which are suggested to be part of a proteolytic cascade initiated by KLK5. To address the roles of KLK5 in NS, we have generated a new transgenic murine model expressing human KLK5 in the granular layer of the epidermis (Tg-*KLK5*). Transgene expression resulted in increased proteolytic activity attributable to KLK5 and its downstream targets KLK7, KLK14 and ELA2. Tg-*KLK5* mice developed an exfoliative erythroderma with scaling, growth delay and hair abnormalities. The skin barrier was defective and the *stratum corneum* was detached *via* desmosomal cleavage. Importantly, Tg-*KLK5* mice displayed cutaneous and systemic hallmarks of severe inflammation and allergy with pruritus. Overtime, persistent scratching led to alopecic, erosive and crusty skin lesions with lymph nodes hyperplasia. The skin showed enhanced expression of inflammatory cytokines and chemokines, infiltration of immune cells, and markers of Th2/Th17/Th22 T cell responses. Moreover, serum IgE and Tslp levels were elevated. Our study identifies KLK5 as an important contributor to the NS proteolytic cascade. Tg-*KLK5* mice help dissecting the complex pathophysiology of NS and and provide a new and viable model for the evaluation of future targeted therapies for NS or related diseases such as atopic dermatitis.

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Functional human epidermal equivalent derived from induced pluripotent stem cells as a model to study genetic correction in Epidermolysis bullosa

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Epidermolysis bullosa (EB) constitutes a group of genetic diseases that result in skin fragility, debilitating blistering and scarring. Recessive dystrophic epidermolysis bullosa (RDEB) is one of the most aggressive forms of EB, caused by absence of normal collagen VII (COL7A1) gene. While significant progress is being made in the field of molecular therapies for RDEB, including gene, protein and cell therapy, a suitable source for efficient replacement of diseased keratinocytes is still lacking. Recent development of induced pluripotent stem cells (iPSCs) that are generated from the somatic cells of individual patients, possess unlimited proliferation potential and are easily amendable for the correction of gene mutation by homologus recombination. Here we demonstrate the generation of the physiologically-relevant model of human epidermis from iPSCs. We have developed a 4 step differentiation protocol to obtain a pure population of iPSC-derived keratinocytes, whose transcriptome corresponds to the gene signature of normal primary human keratinocytes (NHK). Furthermore, following a sequential high-to-low humidity environment in an air-liquid interface culture of iPSC-derived keratinocytes, we were able to generate a functional human epidermal equivalent (HEE) with all cellular strata of the human epidermis and skin barrier with properties similar to normal skin. Such HEE generated from genetically-corrected RDEB-iPSC will be an invaluable tool not only for dissection of molecular mechanisms leading to epidermal barrier defects but will also be instrumental for drug development and screening. Our future goal is to develop a next generation skin stem cell therapeutic for RDEB.

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Optimization of liposomal carriers for RNAi-based topical therapies for skin diseases

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Recently, our group developed ultradeformable SECosomes (Surfactant-Ethanol-Cholesterol containing liposomes) which enabled siRNA delivery in cultured primary skin cells, and human (psoriatic) ex vivo skin. Here, we aim to optimize their transfection and penetration efficiency for their use as topical delivery system for therapeutic RNAi molecules in the treatment of psoriasis and other skin diseases. By altering the 'SEC' formulation, six new liposomes were developed and analyzed as potential RNAi delivery vesicle by examining their size, surface charge, morphology, and encapsulation efficiency. The *in vitro* transfection efficiency of the different liposomes was also tested in psoriasis-induced keratinocytes. Changing the helper lipid or surfactant in the 'SEC' formulation or lowering the ethanol percentage (SEC20) did not result however in major differences. Interestingly, liposomes containing two helper lipids ('DDC') produced favorable vesicles, indicated by their size, homogeneity, morphology, encapsulation efficiency (> 90%) and *in vitro* transfection efficiency. The three best formulations were subjected to *ex vivo* penetration studies in Franz diffusion cells and excised normal and psoriasis human skin. Confocal imaging indicated that in normal human skin the SEC and SEC20 remained restricted to the SC, whereas the DDC liposomes penetrated into the different epidermal layers, without transdermal delivery. As the skin barrier is disrupted in psoriasis, the SEC and SEC20 were able to penetrate throughout the epidermis and rete ridges, comparable to the DDC penetration in psoriatic skin. Currently, DDC complexes containing siRNA against hBD-2 are applied topically to ex vivo psoriatic skin to investigate the therapeutic effect of hBD-2 knockdown. In conclusion, we identified a new promising liposome, 'DDC', capable of delivering RNAi therapeutics to psoriatic and normal human skin, without targeting the dermis and/or circulatory system. These liposomes could be used as topical carrier system for psoriasis as well as for other skin diseases characterized by an intact skin barrier.

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3D-skin models for protein substitution in autosomal recessive congenital ichthyosis

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Here we describe an approach towards personalized protein replacement therapy in autosomal recessive congenital ichthyosis (ARCI). TGase1, 12R-LOX and eLOX3 are crucial enzymes acting in the same pathway important for differentiation of the skin. Mutations in the respective genes lead to an ARCI phenotype with hyperkeratosis, scaling, erythema and impaired skin barrier function. This project focusses on a causative, personalized treatment for ARCI patients substituting the missing enzyme instead of symptomatic treatment, which is the standard management up to now. Therefore we have cloned the genes *TGM1*, *ALOX12B* and *ALOXE3* into GFP- and His-tagged plasmid vectors respectively. FACS-analyses, fluorescence microscopy and western blot have been used to assess mammalian expression systems under various conditions with the GFP-tagged vectors. In a second step His-tagged vectors were used in the optimized protein expression protocol for a larger-scale setup. Obtained cell lysates were then purified by nickel and cobalt NTA beads and TGase1 was tested via ELISA for its specific activity. In parallel we have constructed ARCI 3D-skin models using gene knockdown with siRNA and characterized them regarding epidermal morphology and differentiation by real time-PCR and immunohistochemistry staining. The models are now being used for testing advanced topical drug delivery systems like microneedles and hyaluronic acid. We expect to insert and replace the missing enzymes such as TGase1, 12R-LOX and eLOX3 in order to restore the regular keratinocyte differentiation and epidermal barrier function. Once the principle is established we will be able to adapt this type of therapy also to other known genes involved in the development of congenital ichthyosis.

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A case of pseudoxanthoma elasticum like disorder with multiple coagulation deficiency

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Pseudoxanthoma elasticum like disorder with multiple coagulation deficiency (tentatively named GGCX syndrome) is known to be caused by GGCX (gamma-glutamyl carboxylase) gene mutation. GGCX is an enzyme that carboxylates glutamate residue in a group of proteins including anti-calcification molecules (matrix gla protein(MGP), alpha-fetuin, etc), and vitamine K dependent coagulation factors. We report a 55 years old Japanese male with GGCX syndrome shows pseudoxanthoma (PXE)-like phenotype such as cutis laxa, calcification of elastic fibers in the dermis, peripapillary atrophy of the retina, and coagulation deficiency. Furthermore he has been suffered from dysarthria and motor ataxia suspect of spinocerebellar degeneration as unreported phenotype. We found homozygous deletion of T within C-terminal end. It produces frameshift and yields 77 amino acid larger molecule of GGCX. Abnormal GGCX molecule located in rER in patient fibroblasts, which was similar pattern in normal fibroblasts. However one of GGCX substrate MGP was under carboxylated demonstrated by western blotting using carboxylation dependent antibody to MGP. These data suggest that this mutation induced loss of function of GGCX resulted in elastic fiber calcification as well as coagulation deficiency. The reason why he had symptoms due to possible spinocerebellar degeneration remains to be elucidated.

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A common atopy-associated variant in the Th2 cytokine locus control region impacts transcriptional regulation and alters SMAD3 and SP1 binding

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Type 2 immune responses play major pathogenic roles in atopic diseases. They are directed by Th2 cells and characterized by the signature cytokines IL4, IL5 and IL13. Single nucleotide polymorphisms in the human Th2 cytokine locus and variants in an intronic region of the DNA-repair gene RAD50, including the RAD50 DNase1 hypersensitive site 7 (RHS7), have been associated with atopic traits in genome-wide association studies. This study aimed to characterize the functional impact of the common atopy associated polymorphism rs2240032 located in the human RHS7 on cis-regulatory activity and on differentially binding transcription factors (TF). TF binding was analyzed by electrophoretic mobility shift assays with Jurkat T cell nuclear extracts. TF identification was performed by mass spectrometry. Reporter vector constructs carrying the major or minor allele of rs2240032 were tested for regulating transcription activity in Jurkat and HeLa cells. Allele-specific binding of SMAD3, SP1 and additional putative protein complex partners was identified at rs2240032. The atopy risk allele exhibited decreased SMAD3 and SP1 binding and showed significantly enhanced promoter activity compared to the non-risk allele in Jurkat cells. rs2240032 is located in an RHS7 subunit which itself encompasses repressor activity and might be important for the fine-tuning of transcription regulation within this region. The human RHS7 contributes to the regulation of gene transcription, and the atopy-associated polymorphism rs2240032 impacts transcriptional activity and TF binding.

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A composite enhancer regulates p63 gene expression in epidermal morphogenesis and in keratinocyte differentiation by multiple mechanisms

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p63, a master regulator of keratinocyte gene expression, is the first epidermal marker to be expressed in the surface ectoderm during embryonic development, and becomes progressively confined to the basal proliferative layer of the developing epidermis. The mechanisms that mediate *p63* expression in the developing skin and in epidermal differentiation are still poorly understood. Here we report the identification of a novel genomic regulatory element that, combined with the previously characterized C40 element, constitutes a keratinocyte-specific long-range enhancer (p63LRE). Consistent with their function, both elements are in an open and active chromatin state in keratinocytes and in embryonic epidermis. The genomic region encompassing the two elements drives strong and basal cell-specific expression in mouse embryonic and newborn epidermis, with a p63-dependent mechanism as determined in a *p63*-null background. While p63 sustains its own transcription through the p63LRE element, a search for other transcriptional regulators revealed that a subset of transcription factors involved in commitment to differentiation acts as repressor of p63 transcription in differentiated keratinocytes.

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A newly identified missense mutation of the EDA1 gene in a Hungarian patient with Christ–Siemens–Touraine syndrome

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Christ–Siemens–Touraine syndrome (CST; OMIM 305100) belongs to the group of ectodermal dysplasias and is characterized by the development of sparse hair, abnormal or missing teeth and sweating deficiency. CST is the consequence of mutations located in the ectodysplasin A (EDA1) gene. We have identified a 35-year-old Hungarian man with characteristic dysmorphic facial features, sparse hair, reduced sweating and missing teeth. Direct sequencing of the coding regions revealed a novel missense mutation in the eighth exon (c.971T/A, p.Val324Glu). The affected patient carries the mutation in a hemizygous form. Previous studies reported the association of missense mutations with non-syndromic tooth agenesis. However, the reported hemizygous patient exhibits hypodontia as well as hypotrichosis and reduced sweating. His daughter, an obligate heterozygous carrier of the identified missense mutation, exhibits only mild teeth abnormalities. As the novel missense mutation is located within the tumor necrosis factor (TNF) domain of the ectodysplasin protein, we hypothesize that this genetic variant affects the ectodysplasin/NFκB signaling pathway.

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A novel postzygotic *KRAS* mutation in a Japanese case of epidermal nevus syndrome presenting with two distinct clinical features: keratinocytic epidermal nevi and sebaceous nevi

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Epidermal nevus syndrome (ENS) refers to a heterogeneous group of congenital disorders characterized by the presence of epidermal nevi associated with systemic involvement. Keratinocytic epidermal nevus syndrome and nevus sebaceous/Schimmelpenning syndrome are included in this group. Recently, keratinocytic epidermal nevus (KEN) and sebaceous nevus (SN) were found to be caused by postzygotic mutations in several genes related to cell proliferation, among which are HRAS and KRAS. Interestingly, the same point mutation was reported in KEN and SN. An intriguing question is whether or not KEN and SN are the same disease. We report a case of a two-year-old Japanese girl with two types of nevi on the left side of her body following Blaschko lines present since birth: yellowish plaques suggesting SN on the head and face, and dark brownish verrucous plaques suggesting KEN on the trunk and lower extremity. Detailed examination revealed delayed eruption of the left teeth and a left cerebral arachnoid cyst. Mutation analysis showed a KRAS c.34G>T p.G12C point mutation in genomic DNA derived from her lesional epidermis but not in her nonlesional epidermis or peripheral blood leukocytes. This KRAS mutation has been reported in various carcinomas and sarcomas, but to the best of our knowledge, it has never been reported in KEN, SN or ENS. Keratinocytic epidermal nevus syndrome and nevus sebaceous syndrome have been considered to be different diseases because of differences in clinical features and frequency of systemic involvements. However, our case strongly suggests that both KEN and SN, which are within an identical disease spectrum can be induced by the same KRAS mutation. Clinical feature variation may depend on the developmental stages at which the postzygotic mutation occurred and/or anatomical characteristics of the affected areas.

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A weighted polygenic risk score using 14 known susceptibility variants to estimate risk and age onset of type-I psoriasis

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The purpose is to establish genetic prediction models and evaluate its discriminatory ability in psoriasis in Han Chinese population. We built the genetic prediction models through weighted polygenic risk score(PRS) and confidence interval-based modeling using 14 susceptibility variants in 7,347 samples. The predictive ability was evaluated by receiver operating characteristic analysis. The probability of psoriasis age onset was estimated using Cox proportional hazard models. We found the risk of psoriasis among individuals in the top quartile of the PRS was significantly larger than those in the lowest quartile of PRS (OR = 28.20, 95%CI: 22.95 - 34.95, $P < 2.0\times10^{-16}$). We also observed statistically significant associations between the PRS, family history and early age onset of psoriasis. We also built a predictive model with all 14 known susceptibility variants and alcohol consumption, which achieved an area under the curve statistic of ~ 0.88. Our study suggests that 14 psoriasis known susceptibility loci have the discriminating potential, as is also associated with family history and age of onset. This is the genetic predictive model in psoriasis with the largest accuracy to date.

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Alteration of epidermal ceramides in a patient with Dorfman-Chanarin syndrome and a 15,935 bp deletion of *ABHD5*

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ABHD5 is an activator of the ATGL lipase. *ABHD5* mutations induce ichthyosiform erythroderma and an intracellular accumulation of lipid droplets known as Dorfman-Chanarin syndrome (DCS). We report an unusual large deletion of *ABHD5* in a new patient with DCS and studied ceramides in his skin. A Moroccan with ichthyosiform erythroderma and lipid droplet in leukocytes was studied. *ABHD5* gene and transcript were analysed. Skin of the patient was examined by electron microscopy (EM) and immunofluorescence (IF) using monoclonal antibodies against ABHD5 and ceramides. Analysis of patient *ABHD5* mRNA demonstrated no amplicon by RT-PCR and RT-qPCR. PCR using genomic DNA revealed no amplicons for exons 1 and 2. Using regularly distributed primer pairs from intron 2 to -40,391 bp we detected a large deletion of 15,935 bp and an insertion of 46 bp between the borders. Bioinformatic analysis of the breakpoints revealed presence of LINE-1PA4 and Alu-Sb1 sequences; the 46 bp insertion had 98% of homology with an Alu-Sb1 sequence. EM analysis of skin showed cytoplasmic vacuoles in keratinocytes, melanocytes and fibroblasts. ABHD5 protein was absent in the sebaceous gland in the patient's skin but present in control skin by IF. Ceramides were detected in stratum corneum (SC) of normal skin but lacking in the patient's skin by IF. To date, this deletion is the largest described in the literature among 32 distinct mutations and could be related to a non homologous recombination between LINE-1 and Alu repeats. This deletion results in the total absence of ABHD5 mRNA and protein, leading to a lack of some ceramides in the SC observed by IF. This is in accordance with recent data using thin-layer chromatography and mass spectrometry imaging. In conclusion we report a new DCS patient with an original deletion of *ABHD5* and changes of epidermal ceramides which could be involved in the pathophysiology of this type of ichthyosis.

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***CARD14* Mutations in Familial and Sporadic Pityriasis Rubra Pilaris: Activation of NF-kappaB**

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Pityriasis rubra pilaris (PRP), a papulo-squamous inflammatory skin disorder, is in most cases sporadic, but a familial form, <6% of cases, with autosomal dominant inheritance with partial penetrance has been reported. We recently demonstrated that patients with familial PRP harbor gain-of-function mutations in the *CARD14* gene, an activator of NF-κB. This study investigates whether mutations in this gene also underlie cases of sporadic PRP. DNA from 48 confirmed PRP patients with no family history was used for amplification of exons 3 and 4 of *CARD14* which harbor mutations in familial PRP. The remaining 18 exons were analyzed in a subset of 20 patients with a definitive diagnosis of PRP, also confirmed by clinical and histopathologic features. Among the 16 sequence variants detected, 3 potentially pathogenic mutations together with the mutations identified in familial PRP, were analyzed on the activation of NF-κB. *In vitro* assay was performed in transgenic HeLa cells constitutively expressing a luciferase reporter under a NF-κB responsive element. This approach indicated that all four *CARD14* mutations in familial PRP and one *CARD14* mutation in sporadic PRP activated NF-κB. Thus, while NF-κB activation may be a common mechanism in familial PRP, *CARD14* mutations are present, yet rare, in sporadic cases. To identify the genetic basis for sporadic PRP, we selected four cases, with characteristic clinical presentation and histopathology but without identifiable *CARD14* mutations, for whole exome sequencing. Over 4000 sequence variants were called based on the hg19 reference genome using the Annovar software tool. Among them, 15 sequence variants have allele frequencies <5% in the 1000 Genomes Project and in the NHLBI Exome Sequencing Project. Of these, three are nonsynonymous in 3 different genes, suggesting a role in the pathogenesis of PRP.

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Characterization of a novel mutation in Peeling skin disease and insights into the inflammatory component of the disease

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Generalised peeling skin disease (PSD, MIM 270300) is a rare autosomal-recessive form of congenital ichthyosis. The disease manifests shortly after birth with pronounced erythroderma. In addition, affected individuals develop a generalised, inflammatory, pruritic patchy peeling of the skin. Recently the loss of corneodesmosin (Cdsn) in the superficial layers of the epidermis, caused by nonsense mutations of the *CDSN* gene, was identified to be responsible for the disease. In the present study we analysed the so far unpublished mutation I.345SfsX121 in a compound heterozygous patient suffering from PSD, whose second mutation was p.Lys59X. *CDSN* gene expression was analysed using qRT-PCR and shows decreased expression levels. Immunohistochemistry revealed a residual Cdsn expression, western blot analysis suggested presence of a truncated protein in the epidermis. To get further insights into the pathophysiology of the epidermis in PSD we examined expression levels of caspase-1 (CASP1) and IL18, which showed elevated expression levels in keratinocytes. Our results suggest nonsense–mediated mRNA decay of a truncated Cdsn form. This *CDSN* down-regulation is contrary to another previously characterized mutation which showed elevated expression levels in PSD. The increased expression of CASP1 and IL18 in Cdsn deficiency may be a key feature of the inflammatory component, which correlates with the clinical symptoms of the patients, who suffer from severe pruritus and atopic manifestations.

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Comparative transcriptome analysis in rosacea subtypes display features of the same disease complex without conclusive consecutive evolution

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Rosacea is a chronic inflammatory skin disease of poorly understood origin. Based on its characteristic clinical features (flushing, erythema, burning, chronic inflammation, fibrosis, glandular hyperplasia) and trigger factors, a complex pathobiology involving dysregulation in the immune, vascular, and nervous system can be anticipated. To identify the distinct and commonly dysregulated genes in the different rosacea subtypes, we analyzed whole-transcriptome expression profiles in patients with erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea (PhR) and compared with non-lesional skin of same patient or healthy volunteers. In ETR, dysregulated lipid metabolism and activation of the innate immune system represent the most dysfunctional gene groups, whereas PPR patients display predominantly a complex activation of multiple pathways of innate and adaptive immunity. PhR patients revealed many similarities with PPR gene analyses, but additional genes involved in tissue remodeling in association to inflammation were significantly elevated. In contrast, epidermal growth factor and Wnt signaling family members show diminished expression profiles. Strikingly, comparison of ETR, PPR, and PhR with non-lesional skin or healthy volunteers reveals dysregulation within the same identical gene sets in 49-72% of all altered genes, depending on rosacea subtype. Thus, the three rosacea subtypes are closely related manifestations of the same disease complex; however, conclusions drawn from our data do not support a direct linear evolution of the disease (e.g., beginning with ETR, development to PPR, and finally occurrence of PhR). This study is the most comprehensive transcriptome analysis of rosacea pathophysiology to date and highlights several new candidates for possible therapeutic interventions.

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Correction of type VII collagen in dystrophic epidermolysis bullosa by novel *trans*-splicing molecules

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Premature termination codons in the *COL7A1* gene lead to absence of type VII collagen and anchoring fibrils in the dermal-epidermal junction (DEJ) of the skin, causing severe autosomal recessive dystrophic epidermolysis bullosa (RDEB). The phenotype of this skin disease reveals severe blistering after mechanic trauma, that significantly affects the quality of life. As a causative therapy approach, we use spliceosome-mediated RNA *trans*-splicing (SMaRT), where artificially designed RNA *trans*-splicing molecules (RTMs) specifically replace distinct exons during the natural occurring splicing process. In this study we examine the functionality of the *trans*-splicing technology using a murine RDEB model. To correct a murine *Col7a1* hypomorphic mouse cell line, which expresses only 10% of normal type VII collagen, a 5′ RTM exchanging *Col7a1* exons 1 to 15 was designed. In order to optimize the efficiency of 5′ *trans*-splicing, we specifically focused on the design of the RTM's binding domain. We developed a GFP screening system which allows us to measure *trans*-splicing efficiencies in flow cytometry. With this system we selected highly efficient binding domains covering the endogenous *Col7a1* exon/intron junction. For further optimization we eliminated in-frame termination codons within the binding domain, showing prevention of background expression of unspliced RTM in western blot analysis. Further, we were able to show accurate *trans*-splicing by increased expression levels of endogenous *Col7a1* transcript in qPCR analysis as well as by elevated collagen VII protein expression in western blot analysis. In this study we show that RNA *trans*-splicing is a promising tool for exon replacement and can be significantly improved in terms of efficiency and safety while sustaining full functionality. The obtained data are of importance for future clinical applications of this technology.

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Detection of common mutations in sporadic primary localized cutaneous amyloidosis by DNA mass spectrometry

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Primary localized cutaneous amyloidosis (PLCA) is a relatively common itchy skin disorder in South America and Southeast Asia. The disease is characterized histologically by focal deposition of amyloid in the dermal papillae of lesional skin resulting in severe itching and discolored macules or papules. The precise pathogenesis of PLCA is unclear, but it is considered to be multifactorial, involving both genetic and environmental contributions. The mutations of the genes for the components of the interleukin-31 (IL-31) receptor, *OSMR*β and *IL31RA*, were associated with patients of familial PLCA from previous whole-genome scan and candidate gene studies. To examine if the same mutations could also be found in sporadic cases of PLCA, we aimed to develop a mutation screen panel covering all known *OSMR*β and *IL31RA* mutations in patients with PLCA. Archival skin biopsy specimens from 360 sporadic PLCA patients were recruited for the analysis. DNA extracted from formalin-fixed paraffin-embedded skin samples were analyzed by a new single base extension reaction with matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) technology. Among the 360 sporadic PLCA cases, 5 were identified with p.K697T mutation, 4 with p.P694L mutation, one with p.I691T mutation, and one with p.V631L mutation of the OSMR gene. Furthermore, one case was found to be positive with the IL31RA-S521F mutation. Together, 3.3% (12/360) of the PLCA was confirmed positive with a definitive genetic diagnosis. The average age at onset was earlier in PLCA patients carrying OSMR or IL31RA mutations than that in patients without a definitive genetic diagnosis (31.3 vs. 46.5 years). Our results confirmed the genetic factor in PLCA patients and identified the prevalent mutations from a retrospective study. This finding may have important implications in clinical practice as a molecular diagnostic tool of the disease.

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Disease Spectrum of Ichthyosis with Confetti

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The aim of this study was to investigate clinical and genetic details of a substantial number of ichthyosis with confetti (IWC) patients in order to define major and minor criteria for diagnosis of this rare disease. IWC is a genodermatosis caused by dominant negative mutations in the *keratin 10* gene (*KRT10*). Small heterozygous deletions, insertions, or duplications within *KRT10* lead to a frameshift and consequently to an arginine-rich C-terminus. Patients are born as collodion babies or with a generalized extensive erythema. During childhood numerous confetti-like patches of healthy skin begin to form. The adult cutaneous manifestation of IWC is a generalized scaly erythroderma interspersed with hundreds to thousands of confetti-like patches of healthy skin, where the pathologic mutation appears to be replaced by the wild type sequence through copy neutral LOH. Parallel clinical investigation of six IWC patients revealed a novel spectrum of phenotypes. Several features qualified as major criteria for diagnosis. Most importantly, they include malformation of ears, hypoplasia of mammillae, and dorsal acral hypertrichosis. Direct sequencing revealed different frameshift mutations in intron 6 or exon 7 of *KRT10* in each patient. Here we present a phenotypic spectrum of IWC in six patients with confirmed mutation. Major and minor criteria deduced from these observations will improve diagnosis of IWC. Ectodermal malformations, which are present in all patients, suggest novel classification of IWC as a syndrome.

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Dominant and recessive Olmsted syndrome with erythromelalgia due to *TRPV3* mutations

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Olmsted syndrome (OS) is a rare keratinizing disorder characterised by extensive palmoplantar keratoderma (PPK) with clinical and genetic heterogeneity. Recently, *TRPV3* (transient receptor potential vanilloid 3) missense mutations were identified in dominant and recessive OS. We report one sporadic and two familial cases of severe, atypical OS caused by new *TRPV3* mutations. Our patients presented with progressive and severe nonmutilating PPK associated with intense erythromelalgia and no periorificial keratotic plaques. Exome sequencing identified a *de novo* p.Leu673Phe heterozygous missense mutation in the sporadic patient, a paternal p.Gly568Cys missense mutation, and a maternal splicing-site mutation within *TRPV3* in the two affected brothers. Exome sequencing showed no mutation in *SCN9A*. The p.Leu673Phe and p.Gly568Cys mutations are located in the C-terminal region and the linker region between transmembrane helix 4 and 5 respectively. They involve highly conserved residues across species and are predicted to be damaging by *in silico* analysis. Structural data suggest less drastic consequences of the p.Gly568Cys mutation on channel function, consistent with its recessive inheritance pattern. The splicing-site mutation leads to in-frame skipping of exon 7, removing almost completely the third ankyrin repeat in the N-terminal domain (p.Gln216_Gly262del). The description of three new mutations, including the first defect in the N-terminal region, further implicates *TRPV3* as a major gene in OS. The association with erythromelalgia expands the spectrum of clinical manifestations associated with *TRPV3* mutations.

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Withdrawn

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Founder mutation in epidermal dystonin underlying autosomal recessive epidermolysis bullosa simplex in Kuwait

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Mutations in *DST-e*, encoding epidermal dystonin, also known as the 230-kDa bullous pemphigoid antigen (BP230), underlie some cases of autosomal recessive epidermolysis bullosa simplex (EBS). Thus far, 2 pathogenic homozygous nonsense mutations in *DST-e* have been reported; the affected pedigrees were Kuwaiti and Iranian. In this study, we identified 4 additional, seemingly unrelated, Kuwaiti families in which a total of 7 individuals had similar, predominantly acral, trauma-induced blistering since infancy, and skin biopsy evidence for a complete absence of BP230 immunoreactivity. We performed mutation analysis of *DST-e* and found that all affected individuals were homozygous for the mutation p.Gln1124*, the same mutation that was identified in the originally reported family from Kuwait. In addition, haplotype analysis defined a shared ancestral mutant *DST-e* allele among these 4 families and the previously reported Kuwaiti individual. Clinically, blistering in the homozygotes was life-long and most heterozygotes had no clinical abnormalities. However, one heterozygote had mild transient skin fragility during childhood, an observation noted in the previously reported Iranian pedigree, suggesting that the condition also may be semi-dominant in some pedigrees rather than purely autosomal recessive. In summary, we have identified 4 further EBS families from Kuwait in whom skin fragility is caused by a recurrent nonsense mutation in *DST-e*, with implications for genotype-phenotype correlation as well as mutation detection strategies in this population.

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Generation of induced Pluripotent Stem Cells (iPSCs) from Lymphedema Distichiasis Syndrome (LDS) for Establishment of Human Disease Model.

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Lymphedema-distichiasis syndrome (LDS, OMIM 153400) is a rare autosomal dominant disorder caused by the mutation in the *FOXC2* gene, a family member of forkhead transcription factors. LDS is characterized by early or late-onset congenital lymphedema and distichiasis, *i.e.* multiple rows of aberrant eyelashes. Since FOXC2 protein is essential for development of the lymphatic and vascular systems, and also plays an important role in embryonic morphogenesis of several tissues, patients with LDS present not only with lymphedema but also with occasional varicose veins, congenital heart defects and other features. The pathogenesis of LDS is not well-defined, although mouse model has been developed. In 2006, the method for generating human induced pluripotent stem cells (iPSCs) has been established, and disease-specific iPSCs have been recently generated from several human diseases to develop cell and gene-based therapy and human disease model for investigating disease mechanisms. Therefore, we generated disease-specific iPSCs from monocytes isolated from a patient with LDS by exogenous expression of four reprogramming factors using Sendaivirus vector. We confirmed the authenticity of LDS-iPSCs by gene expression analysis of stem cell markers using RT-PCR and immunostaining. The differentiation capability of LDS-iPSCs was analyzed by the formation of embryoid body and teratoma. We are now trying to differentiate wild-type and LDS-iPSC into lymph vessel endothelial cells (LVECs) using several factors, including activin as a mesoderm initiator, BMP4 as an epithelial initiator and VEGFs as important factors for differentiating into endothelial cells.

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Genetic polymorphisms associated to moderate to severe plaque psoriasis

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Psoriasis is a multifactorial chronic disease of the skin. In this study we aim to determine susceptibility genes in patients with chronic moderate to severe plaque psoriasis and compare the analyzed polymorphisms with healthy controls. We analyzed 173 single nucleotide polymorphisms of genes related with psoriasis and other autoimmune diseases in 191 moderate to severe plaque psoriasis patients and 197 healthy controls. All participants are Caucasians and 24% of the patients have psoriatic arthritis. Results of the multivariate logistic regression analysis showed 9 single nucleotide polymorphisms associated with psoriasis: *PTPN22*, *CD226*, *TYK2*, *IL12B*, *IL1A*, *SLC22A4*, *TNFAIP3*, *HLA-C* and *IκBKβ* genes. Comparing patients without psoriatic arthritis (n=145) *versus* controls, we found 8 single nucleotide polymorphisms previously associated with psoriasis and other 7 in *IL18*, *CLMN*, *CTNNA2*, *RNF114*, *IL12B*, *MAP3K1* and *CCHCR1* genes that may be specific for the development of psoriasis in our population. Our findings show the importance of genes related with the immune system for the developing of moderate-to-severe psoriasis.

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Germline mutation in *EGFR* resulting in epithelial inflammation and metabolic abnormalities.

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A 12 month old Eastern European male infant from a consanguineous Roma family presented with multi-organ epithelial inflammation and electrolyte imbalance of unknown aetiology. Clinically, he had skin erosions, dry scale, and alopecia, but later developed a papulopustular rash. He died aged 2.5 years from sustained metabolic abnormalities compounded by skin and chest infections. Collectively, the clinicopathological features suggested a novel genodermatosis. Whole-exome sequencing disclosed a homozygous loss-of-function missense mutation within *EGFR* (c.1283G>A; p.Gly428Asp), encoding the epidermal growth factor receptor (EGFR). Immunolabelling for EGFR in the patient skin showed cytoplasmic rather than membranous localisation, and transfection of a mutant p.Gly428Asp construct into MCF7 cells (that express low endogenous EGFR) demonstrated that mutant EGFR at the plasma membrane was highly unstable and more susceptible to constitutive endocytosis. Upon ligand stimulation (with EGF) the mutant receptor showed a lack of both EGFR phosphorylation and activation of downstream signalling pathways. Microarray analysis of skin RNA identified disturbed terminal differentiation and raised expression levels of several inflammatory/innate immune response transcripts. Together, these results provide strong support for the pathogenicity of the *EGFR* mutation. This new autosomal recessive genetic disorder reflects the protean role of EGFR in human development and tissue homeostasis. The findings also reveal the pathophysiological abnormalities arising from global disruption of EGFR signalling, observations that are relevant to the cutaneous and extra-cutaneous side-effect profile seen in oncology patients receiving EGFR inhibitor drugs or antibody therapies.

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Homozygous missense mutation in *IL36RN* in generalized pustular dermatosis compatible with both AGEP and generalized pustular psoriasis

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Acute generalized pustular eruptions (AGEP) and generalized pustular psoriasis (GPP) show many overlapping clinical features. Here we report a 40 years old man who initially presented with generalized pustules and systemic inflammatory response syndrome 5 days after intake of amoxicillin, suggesting a diagnosis of AGEP due to amoxicillin. Nine months later, a throat infection was followed by a generalized pustular skin reaction with systemic symptoms without prior drug intake. Clinical presentation and histology was compatible with the diagnosis of GPP, and the patient recalled a similar episode 20 years before. Retrospectively, our initial diagnosis of AGEP was therefore called into question, as drug-triggered GPP was now the likely diagnosis. However, based on current classification systems, it remains unclear whether such cases should be diagnosed as AGEP or drug-elicited GPP. Genetic analysis of this patient identified a homozygous mutation in exon 5 (c.C338T:p.S113L) of the *IL36RN* gene. The IL36RN gene encodes the anti-inflammatory IL-36-receptor antagonist, IL-36Ra, which blocks the pro-inflammatory cytokine IL-36. *IL36RN* mutations can lead to uncontrolled IL-36 signaling and enhanced production of IL-6, IL-8, and IL-1 which give rise to pustular eruptions. Recent findings show that *IL36RN* variants are common in GPP without plaque psoriasis and that they can be found in a subset of AGEP. In this case, the *IL36RN* mutation therefore underlies the generalized pustular eruptions and provides the pathogenetic link between the clinically overlapping presentation of AGEP and GPP. It is likely that in our patient stimulation of the immune system, be it by drug hypersensitivity to amoxicillin or throat infection, led to uncontrolled neutrophilic skin inflammation because of deficiency in IL-36RA. Moreover, our case supports the emerging concept that the disease taxonomy of pustular skin eruptions could in future be based on genetic profiling.

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Ichthyosis linearis circumflexa as the only clinical manifestation of Netherton syndrome

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Netherton syndrome (NS) is a rare autosomal recessive keratinization disorder due to SPINK5 mutations that cause absent or, rarely, reduced expression of the serine protease inhibitor LEKTI. NS is characterized by the triad of (i) congenital ichthyosiform erythroderma in most cases associated with ichthyosis linearis circumflexa (ILC), (ii) a pathognomonic hair shaft defect called trichorrhexis invaginata and (iii) atopy manifestations. ILC consists of migratory, annular and polycyclic scaling patches with double-edged scales at the periphery of the lesions. We studied two young siblings who presented, since the first months of life, erythematous areas on the cheeks followed by the appearance of sparse ILC lesions on the face, trunk, buttocks and proximal extremities, with a remitting and relapsing course. Erythroderma at birth, trichorrhexis invaginata and atopic manifestations were absent and IgE levels were normal. LEKTI immunoreactivity was present in the granular layer and hair follicle in patient skin, although markedly weaker than in control skin. In situ zymography indicated a modest increase of the serine protease activity in the epidermis. Subsequent analysis of the SPINK5 sequence revealed compound heterozygosity for the c.1302+4A>T and c.2240+1G>A splice-site mutations. Gene expression studies performed in patient keratinocytes revealed that the mutation combination correlated with a normal pattern of bioactive LEKTI fragments, which however were released at lower levels and with a different relative abundance compared to normal cells. Our results show that ILC can be the only clinical manifestation of NS, resulting from specific SPINK5 mutation combinations that lead to reduced LEKTI expression. They also point to the relevance of SPINK5 mutation analysis to recognize non-classical NS forms and properly counsel the families.

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Internal exon replacement as a promising tool for *COL7A1* RNA repair

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In recessive dystrophic epidermolysis bullosa (RDEB) mutations are located within the gene *COL7A1* encoding for type VII collagen. Truncated or loss of collagen VII protein results in severe blistering of the skin and mucous membrane which are hallmarks of this inherited skin disease. The aim of this work is the replacement of mutated *COL7A1* exons using the combination of 5′ and 3′ RNA *trans*-splicing also called internal exon replacement (IER). The size-associated limitations which are known in gene therapy could be avoided using IER. A double RNA *trans*-splicing molecule (dRTM) includes splicing elements for efficient *trans*-splicing, two binding domains (BD) binding up- and downstream of the mutated gene region and the wildtype coding exon(s) for exchange. We have established a fluorescence reporter-based screening system in order to select highly efficient dRTMs out of a pool of randomly designed individual dRTMs. In this setup the reporter molecule AcGFP was split up into three parts. The internal part of AcGFP was included in the RTM whereas the 5′ and 3′ end of the reporter were cloned into an artificial target molecule containing the mutated gene region of *COL7A1*. Co-transfection experiments in HEK293 cells revealed a highly efficient dRTM capable to induce GFP expression in over 70% of all analyzed cells. We were able to confirm the full restoration of the reporter molecule GFP on protein level by Western blot analysis after IER. This dRTM can then be adapted for further endogenous experiments in RDEB patient cells. We assume that the methodology of internal exon replacement is a promising tool to correct mutations in the gene *COL7A1* involved in the severe blistering skin disease epidermolysis bullosa.

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Lamina lucida cleavage pattern analysis in junctional epidermolysis bullosa: insights that facilitate the diagnosis

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Mutations in genes coding for laminin 332, type XVII collagen and α6β4 integrin cause junctional epidermolysis bullosa (JEB). The cleavage plane (level of tissue separation) is within lamina lucida. The objective of this study is to investigate the value of lamina lucida cleavage pattern analysis in JEB. Skin biopsies of JEB patients (n=24) were analyzed by means of immunofluorescence antigen mapping (IFM). The results were correlated with electron microscopy (EM). Molecular analysis of all cases was performed. Investigation of immunofluorescence patterns in JEB reveals two types of lucidolytic cleavage: a *low* lamina lucida cleavage and a *high* lamina lucida cleavage. Mutations in type XVII collagen or α6β4 integrin cause a high junctional cleavage, while mutations in laminin 332 lead to a low junctional cleavage. An additional clue to diagnosis is that mutations in type XVII collagen lead to loss or reduction of apical-lateral cell staining in the epidermal basal cell layer. These observations increase the insight into the topographic level of blister formation and staining patterns in JEB. The main implication is that IFM allows for easy and straightforward identification of the candidate gene(s), even in cases where staining is not clearly reduced, thus facilitating the diagnosis.

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Lentiviral-mediated gene editing for correction of *COL7A1* deficient RDEB

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Recessive dystrophic epidermolysis bullosa (RDEB) is a rare and debilitating genodermatosis with neonatal onset caused by mutations in the *COL7A1* gene resulting in non-functional type VII collagen protein (C7) leading to severe blistering and tissue separation at the dermal epidermal junction. Here we report the use of zinc finger nucleases (ZFN) to edit genomic *COL7A1* and restore protein expression. ZFNs were designed to bind exon 4 of *COL7A1* where several hot-spot mutations have been reported. ZFNs are typically delivered via DNA or mRNA transfection in most cell types but with very low efficiency in primary keratinocytes (KC). To improve delivery of these DNA-binding motifs, we have subcloned both aspects of the ZFN pairs into a non-integrating lentiviral vector (NILV) allowing for their transient expression in cells, therefore reducing the risk of insertional mutagenesis. The efficiency of editing in KC using the NILV-ZFNs resulted in a 25% modification compared to 12% when using plasmid ZFN transfection. Clonal expansion revealed 20.8% monoallelic and 4.2% biallelic disruption of the gene. This was further confirmed by the detection of C7 protein expression showing a proportional reduction in cells with monoallelic change and absence in cells with biallelic gene editing compared to normal levels positively correlating with *in-vitro* cell migration functional assays. A NILV carrying endogenous dsDNA donor gene repair template sequence was designed and delivered to ZFN-treated KC cells. Sequencing across the ZFN binding site is expected to reveal both monoallelic and biallelic template insertion by targeted homologous recombination. Our results show an improved method for ZFN and template delivery to KC using viral vectors demonstrating the feasibility of a patient-tailored gene editing strategy for the correction of *COL7A1* and restoration of C7 expression.

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LESIONAL PSORIATIC SKIN: A COMPREHENSIVE ANALYSIS OF GENE EXPRESSION

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Psoriasis is an immune-mediated skin condition with a genetic predisposition. Aim of this paper was to provide a comprehensive analysis of the transcriptome in lesional psoriatic skin. Using bioinformatic approaches we analyzed gene expression in the skin samples subjected to microarray (MA) and next generation sequencing study (NGS). Comparative analysis of the named datasets revealed differences in expression of genes that had a role in terminal differentiation and hyperproliferation of epidermal keratinocytes, antimicrobial peptides and protease inhibitors. We also confirmed the elevated expression of the following Th1 cytokines: IL12A, IL12B, IL17A, IL17F and TNF. Notably, upregulation of TNF was reported for a NGS dataset for the first time. Moreover, we described three gene clusters with a coordinated gene expression. One of these clusters, the complex of epidermal differentiation genes was also known as the psoriasis susceptibility locus PSORS4. In addition, the analysis of NGS data revealed more than 60 differentially expressed genes in lesional skin. Interestingly, these 60 genes were differentially expressed in all lesional samples suggesting that this gene set can be used for diagnosing proposes by comparing two paired biopsies of lesional and uninvolved skin taken from the same patient. The comparative analysis of whole-genome data suggests NGS as the most reliable method to characterize gene expression in psoriatic lesional skin. The data analysis revealed several gene sets with coordinated expression. Notably, each of the mentioned gene sets was composed of the genes with a common function. Moreover, we proposed a molecular signature for sorting the samples of lesional and uninvolved skin. The later can be helpful for diagnostic proposes as well as for evaluation of new experimental drugs.

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MAD-AD: A meta-analysis derived atopic dermatitis transcriptome

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Atopic dermatitis (AD) is a common inflammatory skin disease with limited treatment options due to incomplete understanding of its pathogenesis. Transcriptomic profiling of lesional (AL) and non-lesional (ANL) AD skin has been performed by microarray analysis to generate a "genomic disease signature". Although these experiments have shed some light on the pathology of AD, inter-study comparisons reveal large differences in the resulting sets of differentially expressed genes (DEGs), limiting the utility of direct comparisons of DEGs across studies. In this work we applied novel meta-analysis models to combine four AD microarray datasets from published/submitted studies. Raw data was uniformly preprocessed and batch-adjustment was conducted prior to the analysis. Taking into account within-study variation, a fixed-effect model was applied to 110 samples (60 AL and 50 ANL;47 paired), to define a robust disease profile. This resulted in a comprehensive AD transcriptome with 576 DEGs (at FCH>2, adj.p-values<0.05), with 370 up- and 206 down-regulated genes in AL versus ANL samples. We identified 108 new DEGs (57 up, 51 down) significantly enriched for GO-terms related to epidermal surface lipids and fatty acids. Ingenuity Pathway Analysis (IPA) on these new DEGs revealed enrichment for genes related to Granulocyte Adhesion and Diapedesis and Atherosclerosis Signaling.

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Marked intrafamilial variability in dystrophic epidermolysis bullosa caused by dominant and recessive *COL7A1* mutations

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Patients suffering from dominant or recessive dystrophic epidermolysis bullosa (DDEB/RDEB) develop clinical signs ranging from minor to extensive skin blistering with the severest forms being life-threatening. The different subtypes result from mutations in *COL7A1* encoding type VII collagen (C7) that forms anchoring fibrils (AF) at the dermal-epidermal junction. We present the case of two siblings displaying a severe generalized form of DEB contrasting with mild DDEB segregating on their mother's side for several generations. The DDEB relatives had dystrophic toenails and minor blistering on the legs. In contrast, gradually after birth, both siblings developed extensive skin erosions, numerous milia, and marked skin atrophy, with oesophageal stricture, fusion of digits and growth retardation. The older brother died at the age of 8.5 years from cardiomyopathy. Immunofluorescence mapping of the siblings skin showed marked reduction of C7 staining. Ultrastructural examination of the skin of a DDEB relative displayed slightly abnormal AF. In contrast, AF were rudimentary and rare in the two siblings. Sequencing of *COL7A1* revealed a single dominant heterozygous mutation c.6698G>A (p.Gly2233Asp) in exon 84 in the two siblings and in all DDEB tested family members. Analysis of *COL7A1* transcripts led to the identification of a novel deep intronic mutation IVS19+40G>A at the heterozygous state in the two siblings and their healthy father. This latter strengthens a cryptic donor splice site causing retention of the first 37 nucleotides of intron 19, leading to a premature stop codon. Here we show that the p.Gly2233Asp mutation, known to cause a transient form of DDEB, can lead to minor but persistent blister formation at adulthood and that its cosegregation with a newly identified deep intronic recessive mutation causes severe generalized DEB, which elucidates the disease variability observed in this family.

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Modelling, understanding and treating hyperpigmentation phenotype associated to neurofibromatosis type 1 using embryonic stem cells

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Neurofibromatosis type I (NF1) is one of the most common autosomal dominant transmitted neurocutaneous disorder. NF1 is caused by loss-of-function of the tumour suppressor gene NF1 encoding neurofibromin, a negative regulator of RAS proteins. Despite of the wide clinical expression spectrum, NF1 affects essentially cells and tissues derived from neural crest. Hyperpigmentation and "café au lait macules" apparition is one of the characteristic disorder patterns in all patients in the first years of life. To reveal and understand the molecular mechanisms associated with the loss of neurofibromin on pigmentation phenotype, we took advantage that human embryonic stem cells (hESC) possess the capacity to differentiate into a wide variety of cell types including melanocytes. Melanocytes derived from two hESC carrying the same NF1 mutation, leading to a decrease of the native form of neurofibromin expression, presents a hyperproliferative status and a hyperactivation of MAPK pathways and cAMP-mediated protein kinase A activity. These mysregulation correlates with an increased expression of the proteins implicated in the melanogenesis leading to an accumulation of matures melanosomes containing melanin. This hyperpigmentation phenotype could by reverted using inhibitors. Using embryonic stem cells carrying NF1 mutation we have revealed molecular mechanisms leading to an hyperpigmentation phenotype in melanocytes. This finding highlights the possibility of targeting NF1 associated proteins for therapeutic treatment.

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Modulating UVB induced apoptosis in Kindler sydrome keratinocytes

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Kindler syndrome (KS) is a recessively inherited skin disorder caused by mutations in the *FERMT1* gene encoding kindlin-1, a phosphoprotein involved in β1 integrin activation at focal adhesions, cell cycle and stem cell regulation. KS manifests first with skin blistering and photosensitivity, followed by progressive poikiloderma and mucosal involvement. Here, we addressed the mechanisms of photosensitivity in KS by exploring the effects of UVB irradiation on kindlin-1 negative immortalized keratinocytes from patients with KS (KSK) *in vitro*. Compared to normal human keratinocytes (NHK), survival of KSK after UVB irradiation is significantly reduced in a dose dependent manner, due to reduced cell proliferation (~10% less proliferating cells, $p<0.01$) and increased apoptosis (~2.5 fold, $p<0.05$). We analysed factors and pathways known to be involved in UVB response, and found sustained increase in p38 phosphorylation (Pp38, ~2 fold, $p<0.05$) and significantly elevated mRNA levels of TNF-α (~4 fold, $p<0.05$) in KSK compared to NHK. Phosphorylation of ERK1/2 and AKT, as well as gene expression levels of TGFβ1, TGFβ2 and IL1α were comparable to the control cells. To address direct involvement of Pp38 and TNF-α in UVB induced apoptosis in KSK, the cells were treated with the specific inhibitor of p38 phosphorylation, SB203580, and the TNF-α blocking antibody TNFR before UVB irradiation, and apoptosis was measured by TUNEL and caspase activation (ApoOne) assays. These treatments significantly ($p<0.05$) reduced UVB induced apoptosis in KSK from two KS patients. In addition, the plant extract luteolin, which is known to block p38 phosphorylation and is used in topical applications for sensitive skin, had similar effects. Increasing kindlin-1 expression level in kindlin-1 negative KSK to about 10% of normal controls, significantly reduced UVB induced apoptosis, whereas overexpression of kindlin-2 had no effect. These data uncovered a novel role of p38 and TNF-α in the UVB response in KS, thereby identifying them as potential targets for therapeutic interventions.

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Molecular and morphological Characterization of the inflammatory Infiltrate in Rosacea: new Insights into immune Pathophysiology

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Rosacea is a chronic inflammatory skin disease of unknown etiology. Thus, the treatment for rosacea is often only symptomatic and a deeper insight into the exact pathophysiology is needed. Therefore, we performed a detailed transcriptome analysis of relevant genes in rosacea subtypes (erythematotelangiectatic: ETR, papulopustular: PPR, and phymatous: PhR), compared to non-lesional or healthy skin. Here, we focused on the genes involved in innate or adaptive immunity, and characterized the inflammatory infiltrate using immunohistochemistry. In all rosacea subtypes, the T cell marker CD3 was increased on gene level. Using immunohistochemistry and morphometry, we found an increased infiltrate of $CD4^+$ T cells in ETR and PPR, in particular T helper 1 (T_H1) and T helper 17 (T_H17) cells. In contrast, molecular markers for B cell activation were only occasionally increased, and immunohistochemistry confirmed B cells to be only occasionally localized in single patients with PPR or PhR. High expression levels of chemokines and cytokines known to be involved in immune cell recruitment and activation of macrophages and mast cells were also observed. In sum, the immune response in rosacea shows a T_H1/ T_H17 expression profile, although slight differences between each subtype exist. Moreover, B cells are only occasionally observed, indicating different trigger factor leading to rosacea. Of innate immune cells, macrophages and mast cells were abundantly present, neutrophils only in pustules, correlating well with transcriptome data. No differences were found for Langerhans cells, NK cells or basophils in rosacea patients compared to controls. Our data give a better understanding about the underlying immune pathways in the pathophysiology of rosacea that may lead to novel, more specific therapies for this frequent chronic inflammatory skin disease.

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***MSX1* mutation in Witkop syndrome with a new phenotype: a case report of new Syndrome**

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~~Witkop syndrome is a rare autosomal dominant disorder characterized by the absence of several teeth and abnormalities of the nails. This is the first report of a rare genetic tooth and nail syndrome (TNS) diagnosed in a 2.5-year-old boy with a mutation in 3'-UTR of the MSX1 gene associated with the absence of the incisors, early exfoliation of the canines in primary dentition, and toe-nail dysplasia. n clinical examination, all the maxillary and mandibular primary incisors were missing. His parents stated that the primary incisors of their child had not erupted yet. Extraoral examination revealed lip eversion and fine hair, while the eyebrows and eyelashes were normal. No heat intolerance or any inability to sweat was reported. The toenails were spoon-shaped and hypoplastic. Periapical radiography showed primary anterior germs. Regarding the early exfoliation of the primary canines, a diagnostic test was requested to determine the levels of serum alkaline phosphatase and urinary phosphoethanolamine, but no abnormality was reported. Also in this study, we propose a simple AvaII enzyme digestion for the analysis of this particular mutation.The parents of the patient had no dental and nail anomalies. In present case we focused on chief complaints of patient (early exfoliation of primary canine teeth). This finding was not reported in witkop syndrome yet. The full article of this case was printed in Iranian Medical sciences Journal in 2013.

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New modulated genes in psoriasis-derived keratinocyte subpopulations.

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T cells play a crucial role in the pathogenesis of psoriasis, recent data emphasize the key role of keratinocytes that, by carrying intrinsic alterations, could determine the formation of skin lesions resembling psoriasis, without the participation of T-cell derived cytokines. In particular, transit amplifying (TA) cells, the stem cell progeny, seems to be responsible for the psoriatic phenotype. The aim of this study was to analyze the role of keratinocytes sub-populations in the pathogenesis of psoriasis. We analyzed the gene expression profile (GEP) of human keratinocyte sub-populations (stem, "early" TA (ETA) and "late" TA (LTA) cells), derived from healthy skin, lesional and non-lesional psoriatic skin. The total RNA samples, extracted from keratinocyte subpopulations immediately after separation, were hybridized onto the Affymetrix human U133 plus 2.0 GeneChip Array. We identified a small number of up-regulated genes (12 probe sets, corresponding to 8 genes) in keratinocyte subpopulations derived from lesional psoriasis vs. healthy and non-lesional psoriasis. We confirmed the increased expression levels of TCN1, S100A7A, KYNU, SERPINB13, FOXE1, but solely due to the keratinocyte component. We identified for the first time the up-regulation of TMEM171, CLEC7A and NDRG4, which seems to correlate with the pathophysiology of psoriasis. Moreover, GEP analysis of lesional psoriasis sub-populations, as compared to the non-lesional psoriatic counterpart revealed the modulation of 17 probe sets, corresponding to 13 genes. Among these genes, we recognized for the first time the up-regulation of IL13RA1, CCDC109B and CD47. These results indicate the importance of keratinocyte compartment in psoriasis, opening the way to the study of new genes potentially critical in the pathogenesis of psoriasis.

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New transgenic RDEB mouse model using editing of *COL7A1* genomic sequence

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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe genetic skin fragility disorder caused by mutations in the *COL7A1* gene, encoding type VII collagen, which stabilizes the dermal-epidermal junction. There is no specific treatment and several approaches using gene, cell and protein therapies are currently being developed. In order to demonstrate the efficacy of these approaches *in vivo*, viable animal models that recapitulate the human disease are needed. We are developing a new transgenic mouse carrying a recurrent hypomorphic *COL7A1* recessive mutation in exon 80. To engineer selected mutations in the human *COL7A1* genomic sequence, we have established an efficient "scare less" method using genome editing by a two steps homologous recombination strategy on bacterial artificial chromosome. In the first step, the targeting construct 1 contains a selectable *Neo* cassette and homology arms identical to the targeted *COL7A1* sequence but containing the desired mutation. In the second step, the targeting construct 2 contains a non-selectable *COL7A1* fragment which is exchanged with the selectable *COL7A1* sequence from step 1. The clones in which the *COL7A1* DNA is correctly targeted are selected for loss of kanamycin resistance. We could demonstrate integration of the selection cassette into the *COL7A1* gene and its subsequent removal by size shift of DNA fragments obtained by PCR amplification using primers flanking the modified exon. Further sequence analyses confirmed the introduction of the desired mutation and integrity of the genomic sequence near exon 80. A linear fragment containing the entire mutated human *COL7A1* genomic sequence was used to generate transgenic animals. These animals will be characterized and crossed with *Col7a1* knockout mice to generate an RDEB mouse model containing the human *COL7A1* with the desired mutation. Here we demonstrated an efficient strategy to introduce specific mutations in *COL7A1* that can be used to generate new models for *in vivo* testing of relevant therapeutic approaches for RDEB.

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Normal human adipocytes gene expression profiling using a robotic workstation coupled to microarrays

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Adipocytes are involved in several biological mechanisms such as lipid, glucose metabolism and endocrine functions. They also secrete adipokines which act as classic circulating hormones to communicate with other organs. In order to better understand the regulation of gene expression involved in the main molecular pathways of adipocyte metabolism, the objective of this work was to develop an automatized protocol allowing us to quantify gene expression in normal human adipocytes and to study their modulation under different treatment conditions. A custom designer gene card based on the sensitive TLDA technology (TaqMan Low Density Arrays) is used. The gene card contains 22 genes chosen from their known function associated with adipokines, lipid metabolism, and fatty acid oxidation. The microarrays were processed using an automate sample preparation (MicrolabStar, Hamilton) which allowed us to perform RNA extraction, RNA quantification/normalization of the samples and the reverse transcription step. The TLDA microarrays were loaded with the different samples tested in triplicate and used for real-time PCR reaction. Results are expressed in Relative Quantification (RQ). To evaluate the repeatability of our protocol, 4 independent mature adipocyte cultures were performed and each culture was treated for 24 hours with Forskolin 10µM. Forskolin is known to act directly on targets involved in lipolysis and adipogenesis and its effects are compared to basal gene expression obtained from untreated adipocytes. The results show that Forskolin is able to modulate the expression of 15 genes, among them, for example, Adiponectin, MCP-1 and Hormone Sensitive Lipase genes are upregulated and AQP7 and UCP2 genes are downregulated in a repeatable manner and in accordance with the literature. Forskolin is considered as our positive control in this new automatized tool associated with a robust experimental protocol to screen active ingredients for lipolytic and adipogenetic projects in the cosmetic field.

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Novel cryptic splice acceptor site activating mutation in the LAMB3 gene with founder effect causes serial Herlitz-type junctional epidermolysis bullosa

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Recently, we encountered a high incidence of recessive lethal Herlitz-type junctional epidermolysis bullosa (HJEB) in infants born to Hungarian Roma parents residing in a small area. The diagnosis was confirmed in two newborns who lacked functional laminin 332 in the cutaneous basement membrane. Sequencing the genomic DNA, a novel G>A mutation was discovered in Intron 10 of the LAMB3 gene. The mutation activates a cryptic splice acceptor site and leads to the insertion of a short fragment from Intron 10 into the mRNA between Exons 10 and 11. All five of the infants with HJEB studied were homozygous for the mutation and their parents were heterozygous carriers. Sequence analysis of the cutaneous LAMB3 mRNA in one newborn let us define the effects of the abnormal splicing: a Ser-Leu substitution and a hexapeptide inclusion with frameshift and PTC in Exon 11. When we obtained family histories from the affected families, we found that 8 more babies had died of severe skin blistering at early ages. The rate of consanguinity was quite high in this community as was the carrier rate of the mutant allele: among 64 individuals screened, 30 were carriers. Using Y chromosome H1a-M82 haplogrouping and its haplotyping, two male HJEB newborns and 5 fathers from the affected families were found to be Romungro as are majority of the Roma in Hungary. The LAMB3 mutation does not appear to be common in Roma however; it was not found in any of the 306 unrelated Hungarian Roma tested. To determine the age of this founder mutation, we constructed a median joining haplotype network within the H1a-M82 haplogroup. The estimated age of the mutation is 548±222 years (95% CI, 326-770 years calculated with 25 years per generation), while Roma have been in Hungary for about 500-600 years. Mutation screening and genetic counseling are now available to prevent further lethal HJEB cases in the isolated community where we worked.

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Novel *FERMT1* Mutations in Consanguineous Iranian Families with the Kindler Syndrome

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The Kindler syndrome (KS) is a rare autosomal recessive genodermatosis characterized by diffuse poikiloderma, cutaneous atrophy, acrokeratosis, trauma-induced blisters and photosensitivity. Mutations have been previously disclosed in the *FERMT1* gene which encodes kindlin-1, a component of keratinocyte focal adhesions. The incidence of KS is expected to be high in Iran due high rate of consanguineous marriages. We identified a total of 13 Iranian families with KS, including 40 affected individuals. Pedigree analysis of these families suggested high degree of consanguinity. The patients demonstrated characteristic clinical features, while the parents, obligate heterozygote carriers, were clinically unaffected. Mutation analysis of *FERMT1* by amplification of all 15 exons and flanking intronic sequences, followed by bidirectional sequencing, revealed homozygous nonsense mutations, both recurrent and novel, including c. 1176T>G, c.1383C>A, c.550_551insA, [c. 889 A>G,c.1139+2 T>C], c.957+1G>A, c994_995delCA, and c.910G>T. One of the novel mutations is missense, two are splice site mutations and all the remaining five mutations cause premature termination codons. These previously unpublished mutations in the *FERMT1* gene expand the spectrum of the mutations underlying KS. The identified mutation database forms the basis to confirm the clinical diagnosis by genetic testing and to identify heterozygous carriers, coupled with genetic counseling, to reduce the burden of KS in Iran.

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Oxidative stress and mitochondrial alterations in Kindler Syndrome

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Kindler Syndrome (KS) (OMIM 173650) is an autosomal recessive skin disorder caused by mutations in FERMT 1 and characterized by skin blistering, photosensitivity, premature aging and skin cancer predisposition. However, the known functions of FERMT1, involved in cell adhesion, do not suffice to fully understand the pleiotropic nature and clinical variability of this genodermatosis. Some of these pathognomonic features of KS not related to cutaneous fragility, such as premature skin aging and cancer predisposition, have been strongly associated with oxidative stress. Mitochondrial oxidative stress has long been implicated in both, aging and cancer. During oxidative phosphorylation, reactive oxygen species (ROS) are generated as by-products and its accumulation results in DNA damage as well as increased cancer susceptibility. We have explored the potential role of mitochondria-derived oxidative stress on the pathogenesis of KS. For that purpose we studied different biomarkers of oxidative stress in cells from KS patients. Patient-derived keratinocytes showed an altered oxidative status as demonstrated by increased glutathione and malondialdehyde levels and by a highly sensitive chimerical redox biosensor system. The ultrastructural analysis of KS skin biopsies and keratinocytes revealed striking abnormalities in mitochondria. In addition, keratinocytes from KS patients showed a reduced and diffuse MitoTracker Red staining and a significant reduction of the membrane potential. Overall, our data indicate that mitochondria in KS are not only altered in structure, but also in their distribution and functionality. This is the first study to demonstrate that mitochondrial dysfunction and oxidative stress contribute to the etiology of KS.

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Regulation of IL-33 expression by normal human epidermal keratinocyte

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IL-33 is a member of the IL-1 family, and important to adaptive Th2 immunity. We previously reported that IFNγ induced expression of IL-33 in normal human epidermal keratinocytes (NHEKs). On the other hand, it has been reported that IFNγ did not induce IL-33 in monocyte, while TNFα and IL-1β did. Recently mouse IL-33 (mIL33) has been revealed to have two distinct transcripts with different non-coding exon1, both of which code the same protein sequences. We have confirmed that there were three different transcription products of IL-33, each containing one of three distinct exon1s, i.e., exon1a, 1b and 1c. We speculated that each exon1 has its own promoter region, which may be regulated in cell type- and stimulus-dependent manner. We aimed to clarify the differential usage of three distinct promoters containing each exon1 in different types of cells, with various stimuli. We amplified approximately 2.5 kbp region of the 5′ UTR of each three exon1 with PCR and cloned them into upstream of firefly luciferase gene construct. These constructs were transfected to NHEKs, human umbilical endothelial cells (HUVECs) and normal human dermal fibroblasts (NHDFs), stimulated with IFNγ or TNFα, and luciferase assay was performed. The construct containing 5′ UTR of exon1b had high luciferase activity without stimulation in NHEKs, HUVECs, and NHDFs. It strongly responded to IFNγ, but did not respond to other stimuli investigated, such as TNFα, IL-1β and LPS for TLR4 ligand in NHEKs and HUVECs. In NHDFs, exon1b containing promoter construct did not respond to any stimuli investigated. Other constructs containing 5′ UTR of exon1a or exon1c, showed only very weak luciferase activity, which did not respond to any stimuli investigated. We identified an active promoter of hIL33. The regulation of IL-33 transcription in NHEKs, HUVECs, and NHFDs may depend on the same promoter, which may utilize common transcription factors.

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Reversion-triggered release of confined lethal mutations: an unreported genetic pathogenesis

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Loss of gene functions due to nonsense mutations is a typical pathogenic mechanism of hereditary diseases. They may, however, in certain genetic contexts, confine the effects of other dominant pathogenic mutations. Theoretically, reversion of such disease-confining mutation may elicit a disease, like the opening of Pandora's box, although no actual case have been reported. We report the first instance in the literature where the reversion of a "confining" nonsense mutation in *GJB2* gene released the dominant pathogenic effect of a coexisting gain-of-function mutation, eliciting the lethal keratitis-ichthyosis-deafness (KID) syndrome. We encountered a KID syndrome patient who had a heterozygous missense mutation p.Gly45Glu in *GJB2* gene. Unexpectedly, her healthy mother also had the heterozygous missense mutation p.Gly45Glu, as well as another heterozygous nonsense mutation p.Tyr136X (Ogawa *et al.*, PLoS Genet. 2014). The biological relationship between the parents and the child was confirmed by genotyping of 15 short tandem repeat loci. We performed haplotype analysis using 40 SNPs spanning the >39 kbp region surrounding the *GJB2* gene to find that an allelic recombination event involving the maternal allele carrying the mutations generated the pathogenic allele unique to the patient. Previous reports and our mutation screening support that p.Gly45Glu is in complete linkage disequilibrium with p.Tyr136X in the Japanese population. Estimated from statistics in the literature, more thane 10,000 p.Gly45Glu carriers in the Japanese population are protected from the disease by this second-site mutation. The reversion-triggered onset of the disease shown in this study is a previously unreported genetic pathogenesis based on Mendelian inheritance.

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Selection of antisense oligonucleotides for trans-splicing enhancement using a fluorescence-based screening system

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RNA *trans*-splicing is a promising tool for RNA repair in genetic diseases. The RNA *trans*-splicing process is induced by RNA *trans*-splicing molecules (RTMs) via specific binding of the respective target pre-mRNA region. To accelerate RTM design we have established a reporter-based screening system which allows us to analyse the impact of defined factors on the RTM *trans*-splicing efficiency *in vitro*. Using this screening system we are able to analyse the potential of antisense oligonucleotides (ASOs) to improve the *trans*-splicing capacity of a selected RTM, specific for intron 102 of *COL7A1*. Defects in collagen type VII encoded by *COL7A1* are associated with the dystrophic form of the skin blistering disease epidermolysis bullosa (DEB). A selected ASO, which interferes with the competitive splicing elements on a stable expressed *COL7A1*-minigene (*COL7A1*-MG), was co-transfected with a highly functional RTM into HEK293 cells leading to a significant increase of the RNA *trans*-splicing efficiency. Thereby, accurate *trans*-splicing between the RTM and the *COL7A1*-minigene is represented by the restoration of full-length acGFP, quantified on mRNA and protein level. We assume that this observation can improve the RTM-mediated correction of genes on mRNA level, especially in cases where a high *trans*-splicing efficiency is required.

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SNEV$^{hPrp19/hPSO4}$ haploinsufficiency accelerates premature skin aging in response to 8-methoxypsoralen/UVA treatment in mice

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Senescent cells accumulate during aging in various tissues and contribute to organismal aging. However, factors that are involved in the induction of senescence in vivo are still not well understood. SNEV$^{hPrp19/hPSO4}$ is a multifaceted protein, known to be involved in DNA damage repair, including interstrand cross link (ICL) repair, and senescence, albeit only in vitro. In this study we used heterozygous SNEV$^{+/-}$ mice (SNEV knock-out results in early embryonic lethality) and wild type littermate control mice as a model to elucidate the role of SNEV$^{hPrp19/hPSO4}$ in DNA damage repair and senescence in vivo. We performed PUVA treatment, consisting of 8-methoxypsoralen (8-MOP) in combination with UVA on mouse skin to induce DNA damage - prevalently ICL - and premature skin aging. We show that SNEV$^{hPrp19/hPSO4}$ expression decreases during organismal aging, while p16, as marker of aging in vivo, increases. In response to PUVA treatment on the skin of old SNEV$^{+/-}$ mice, we observe increase of γ-H2AX level, as DNA damage marker, accompanied by reduced epidermis thickening, increase of p16 level as well as loss of structure accompanied by increase in MMP1 levels. Thus, the DNA damage response occurring in the mouse skin upon PUVA treatment is dependent on SNEV$^{hPrp19/hPSO4}$ expression and lower levels of SNEV result in accumulation of DNA damage, increase of cellular senescence and thus in acceleration of premature skin aging.

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Splicing regulation disturbances in psoriasis pathogenesis

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In a recently performed cDNA microarray experiment we identified three splicing regulators (serine/arginine-rich splicing factor 18 (SFRS18), peptydilpropyl isomerase G (PPIG), luc-7 like3 (LUC7L3) that where differentially expressed in response to T-lymphokines in healthy and psoriatic non-involved epidermis samples. We and others have previously shown that the oncofetal splice variant of fibronectin - the isoform containing the EDA domain (EDA+) - is overexpressed in psoriatic non-involved epidermis. In this study we investigated whether SFRS18, LUC7L3 and PPIG are able to alter the ratio of the normal (EDA-) and psoriasis-associated (EDA+) variant. To this aim the expressions of the splicing regulators were silenced in immortalized keratinocytes (HPV-KER). We found that the EDA+/EDA- ratio was altered upon silencing of LUC7L3 and PPIG: before silencing the expression level of EDA+ fibronectin isoform was higher than the EDA- fibronectin, but as a result of silencing the amount of the two splice variants became comparable. In addition, the expression patterns of the splicing regulators were compared in two different synchronized, immortalized cell lines, HaCaT and HPV-KER cells. Gene and protein expression patterns of the three regulators were very similar in both cell lines during their proliferation and differentiation suggesting that they may share common regulation.

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Striking intrafamilial phenotypic variability in loricrin keratoderma

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Loricrin keratoderma (LK) is an autosomal dominant disorder of keratinization, characterized by the presence of distinct heterozygous mutations in the loricrin gene (*LOR*). To date, only 11 families with LK have been reported. Common clinical features of LK include diffuse palmoplantar keratoderma (PPK) with honeycomb pattern (HCP), generalized ichthyosis and pseudoainhum. Some LK patients showed erythroderma, clinically diagnosed as having congenital ichthyosiform erythroderma (CIE). One family with LK developed non-migrating hyperkeratotic erythematous plaques, with a clinical diagnosis of progressive symmetrical erythrokeratoderma. Thus, LK patients show wide interfamilial clinical variability. However, the clinical features of LK are not yet fully understood, due to the scarcity of LK families. In this study, we investigated a Japanese family with LK. A proband, a 36-year-old man, exhibited generalized dry, scaly skin from birth. He also showed non-migrating scaly reddish plaques on the chest and limbs. Reticular erythema was evident on his trunk. He showed PPK, but without HCP or pseudoainhum. The absence of family history initially led us to a diagnosis of CIE. Several years later, he fathered two daughters, both of whom also developed generalized mild ichthyosis. Notably, they showed HCP-PPK and mild pseudoainhum. A diagnosis of LK was made by exome and Sanger sequencing, which identified a heterozygous *LOR* mutation, c.545_546insG. Our findings suggest that the absence of HCP-PPK and/or pseudoainhum does not exclude the possibility of LK and that the presence of PPK and generalized ichthyosis indicates a possible diagnosis of LK. Our results also indicate that patients with LK can show striking intrafamilial clinical variability, even when carrying the same mutation. Moreover, we show that LK patients can develop reticular erythema, which had not been described previously. These results provide further phenotypic insights into LK.

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The effect of two endogenous retinoids on the mRNA expression profile in human primary keratinocytes, focusing on genes causing autosomal recessive congenital ichthyosis

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Retinoids (natural forms and synthetic derivatives of vitamin A) are used as therapeutic agents for numerous skin diseases, such as keratinization disorders (e.g. ichthyoses) and psoriasis. Two endogenous ligands for retinoic acid receptors exist, retinoic acid (atRA) and 3,4-didhydroretinoic acid (ddRA). In primary human epidermal keratinocytes many transcriptional targets for atRA are known, whereas the targets for ddRA are unknown. In an attempt to determine the targets we compared the effect of atRA and ddRA on transcriptional profiles in undifferentiated and differentiating keratinocytes. First, as expected, many genes were induced or suppressed in response to keratinocyte differentiation. Furthermore, the two retinoids affected substantially more genes in differentiated keratinocytes (>350) than in proliferating keratinocytes (≈ 20). In differentiating keratinocytes markers of cornification were suppressed suggesting a de-differentiating effect by the two retinoids. When comparing the expression profile of atRA to that of ddRA no differently regulated genes were found. The array analysis also found that a minor number of miRNAs and a large number of non-coding transcripts were changed during differentiation and in response to the two retinoids. Furthermore, the expression of all, except one, genes known to cause autosomal recessive congenital ichthyosis (ARCI) were found to be induced by differentiation. These results comprehensively document that atRA and ddRA exert similar transcriptional changes in keratinocytes and also add new insights into the molecular mechanism influenced by retinoids in the epidermis. Furthermore, it suggests which ARCI patients that could benefit from therapy with retinoids.

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The transcription factor FOSL1 and its role in psoriatic lesional skin

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The gene that encodes the transcription factor FOSL1 is upregulated in lesional psoriatic skin and downregulated in the period of disease remission after the patient finished a course of PUVA. The aim of this study was to explore the role of FOSL1 in psoriasis via identification of FOSL1 target genes in lesional psoriatic skin. Next generation sequencing technique (NGS) was used to identify differentially expressed genes. Immunohistochemistry was used to detect FOSL1 in psoriatic skin lesions. Bioinformatics approaches were used to identify FOSL1 target genes. The ability of RAGE ligands to induce the identified FOSL1 target genes was verified by stimulation of epidermal keratinocytes (HaCaT) with the named proinflammatory factors. The immunohistochemistry results confirmed the activation of FOSL1 in psoriatic skin lesions. The NGS analysis revealed that the following FOSL1 target genes, namely CCL2, CCND1, FOSL1, HMOX1, IL8, IVL, MGP, MMP1, MMP9, and PLAUR were differentially expressed in the plaques. In addition, we were able to induce some of the named genes by a treatment of HaCaT with the proinflammatory factors S100A7-S100A7 and S100A8-S100A9. We conclude that the transcriptional regulator FOSL1 can be involved in pathogenesis of psoriasis for two reasons. First, the certain FOSL1 genes were differentially expressed in lesional skin. Second, the proinflammatory factors, namely S100A7-S100A7 and S100A8-S100A9 dimers were able to induce the certain FOSL1 target genes the *in vitro* model that was often used to imitate of human hyperproliferative epidermises. We suggest targeting FOSL1 and FOSL1-controlled genes as a new therapeutic approach for psoriasis.

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The up regulation of ABCA1 by the activation of LXRβ and PPARδ/γ to renew archetypical differentiation in Harlequin Ichthyosis

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Harlequin Ichthyosis (HI) is the most severe and frequently fatal form of congenital ichthyosis. In 2005, we discovered that recessive mutations in ABCA12, a keratinocyte transporter, were causative in HI. Loss of ABCA12 leads to defective transport of glucosylceramide into lamellar bodies, impaired transportation of proteases, premature terminal differentiation and retention of squames, leading to skin with thick hyperkeratotic plates characteristic of the HI phenotype. We have found via gene expression array data, that transcription of the cholesterol transporter ABCA1 is down regulated after ABCA12 shRNA ablation of transcript. Additionally, through Ingenuity Pathway Analysis we hypothesized that the down regulation of ABCA1 may be due to a dysregulation of the nonsteroidal nuclear hormone receptors, the liver X receptor (LXR) and the peroxisome proliferators-activated receptors (PPARs). Activation of LXRβ and PPARδ/γ has been previously shown to increase transcription of ABCA1. We confirmed the down regulation of ABCA1, LXRβ and PPARδ/γ using immunohistochemical analysis of a HI patient biopsy and western blot analysis of keratinocytes that had undergone ABCA12 siRNA knockdown. We have developed and characterised a HI cell line, which displays terminal differentiation markers prematurely under calcium shift and in the organotypic 3D cultures, which also presented with a thickened stratum corneum. The application of PPARδ/γ and even more so LXRβ agonists facilitate an increase of ABCA1 expression in the patient derived HI cell line. These possible therapeutic strategies could aid cholesterol transportation in the epidermis, crucial in permeability barrier formation and in turn the movement of desquamation proteases to the stratum corneum.

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Transcriptome Analysis of Psoriatic Epidermis

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Split thickness skin grafts (STSG), consisting mainly of the epidermis and only a small amount of dermis, were taken from 9 healthy control (C), 5 psoriatic non-lesional (PN), and 6 lesional (PL) patients and subjected to RNA 5′-end sequencing. Total RNA per cell in PL was higher than in the C and PN samples; the data, therefore, was analyzed by using RNA spike-in normalization instead of global normalization. We found only 35 differentially expressed transcripts (DETs) in the PN skin when compared with the controls (FDR < 0.05). 28 of these were up-regulated (FC > 1.5) and only 7 down-regulated (FC < 0.75). Interestingly, 12 of the DETs map to the known *PSORS* loci, of which *PSORS4* was the most represented among the up-regulated ones. 2437 and 3541 transcripts were up-regulated and 2550 and 491 down-regulated in the PL, when compared with the C and PN samples, respectively. Gene set enrichment analysis with the differentially expressed genes (DEG) in PL, revealed enrichment at the *PSORS4* locus. The functional annotation analysis of DEGs highlighted gene ontology groups related to keratinocyte and epidermal cell differentiation, already in the PN samples. Furthermore, PL-DEGs showed enrichment in many of the functional groups previously associated with psoriasis: e.g. innate immunity and cell adhesion. Comparison of our STSG DEGs with the previous gene expression data from full thickness (FT) psoriatic samples shows considerably less down-regulation, which may be due to the high amount of dermis in the FT samples; the expansion of epidermis in psoriasis leads to decrease in dermal genes. These results indicate that our samples enable direct focusing mainly on the epidermal transcripts. RNA-Seq also enables the identification of different start sites and transcripts from the introns and untranslated regions. In addition, the sensibility allows a more concrete recognition of the altered signaling pathways.

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Ultrastructural abnormalities in exfoliative ichthyosis with a novel mutation in *CSTA*

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Recently, mutations in CSTA, encoding the cysteine protease inhibitor A, which seems to be critical in desmosome-mediated cell-cell adhesion within the lower epidermal layers, were identified as the genetic cause of autosomal-recessive exfoliative ichthyosis (AREI). AREI is characterized by diffuse palmoplantar hyperkeratosis and coarse skin peeling of the hands, feet and distal extremities without blisters, and often mediated by humidity. We performed ultrastructural analysis by transmission electron microscopy, including ruthenium tetroxide postfixation in a 25-year-old patient with AREI, who exhibited the unknown homozygous loss-of-function mutation c.172C>T (p.Arg58Ter) in CSTA. Ultrastructural features included normal degradation of corneodesmosomes, mild intercellular oedema in the spinous layer but not in the basal layer, normal appearing desmosomes, and prominent keratin filaments within the basal keratinocytes. Thickness of cornified envelopes was reduced, and epidermal barrier abnormalities were characterized by abnormal lamellar lipid bilayers in the stratum corneum, with non-lamellar electron-dense material and vesicular contents, premature and inhomogeneous lamellar body secretion and delayed processing of secreted lamellar body contents. Barrier abnormalities were reminiscent, albeit less severe, of Netherton syndrome (NS), which results from deficiency of serine protease inhibitor LEKTI. Accelerated proteolysis of corneodesmosomes in NS is caused by an imbalance in kallikreins and leads to ichthyosiform erythroderma and atopic manifestations. This work expands the morphological features and provides evidence of epidermal barrier abnormalities in AREI.

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Whole-exome sequencing reveals genetic modifiers associated with elevated IgE and allergic sensitization in ichthyosis

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The crucial role of the skin as a barrier is mainly dependent on terminally differentiated keratinocytes, the corneocytes, which are dynamically and regularly desquamated, so that the stratum corneum becomes repopulated by new cells. Numerous molecular players govern these processes, and mutations in the corresponding genes result in ichthyoses, characterized by generalized skin scaling. Using whole-exome sequencing, we performed a comprehensive genetic analysis in a young male displaying a keratinization disorder with episodes of dermatitis, allergic sensitizations and high IgE levels, without specific histopathologic and ultrastructural features. We show that besides the *NIPAL4* mutation c.527C>A, p.A176D which caused the ichthyosis, additional disease modifying variants in the genes encoding LEKTI and filaggrin contribute to the distinct allergic manifestations. Importantly, although the variants, p.E420K and p.S368N in LEKTI, and p.P478S in filaggrin, have been associated with atopic dermatitis in large-scale studies, they did not cause any cutaneous disease in the parents or siblings of our patient, but became relevant modifiers only in the context of the major keratinization defect present in the patient. Our findings support the idea that a complex interplay exists between mutations and functional variants in genes for proteins involved in epidermal differentiation, resulting in a spectrum of barrier function defects and allergic manifestations.